LESSON 16:
OVARY/ OVULE CULTURE

Objective

Ovary/ ovule culture is another method used for the production of Haploid plants. Since, the haploids are important in plant breeding (already given in previous Lesson), methods have been devised to obtain them in cultures. The objective of this Lesson is to study ovary/ ovule culture which is an alternate method for haploid production.

How is this achieved?

This can be achieved by culture of unfertilized ovaries to obtain haploid plants from egg cell or other haploid cells of the embryo sac. This is called ovary culture, and the process is termed as gynogenesis.

The first report of gynogenesis was by San Noem in 1976 in case of barley. Subsequently, success has been obtained with many species, e.g., wheat, rice, maize, tobacco, petunia, Gerbera, sunflower, sugarbeet, rubber etc.

About 0.2-6% of the cultured ovaries show gynogenesis and one or two, rarely up to 8, plantlets originate from each ovary. The rate of success varies considerably with:

1. Species and
2. Markedly influenced by the genotype so that some cultivars do not respond at all. In rice, japonica genotypes are far more responsive than indica cultivars.
3. Stage of ovary development:

   In most cases, the optimum stage for ovary culture is the nearly mature embryo sac, but in rice ovaries at free nuclear embryo sac stage are the most responsive. Generally, culture of whole flowers, ovary and ovules attached to placenta respond better, but in Gerbera and sunflower isolated ovules show better response. Cold pretreatment (24-48 hr at 4°C in sunflower and 24 hr at 7°C in rice) of the inflorescence before ovary culture enhances gynogenesis.
4. Growth Regulators: GRs are crucial in gynogenesis, and at higher levels they may induce callusing of somatic tissues and even suppress gynogenesis. GR requirement seems to depend on species. For example, in sunflower, GR-free medium is the best, while even at low level MCPA (2-methyl-4-chlorophenoxyacetic acid) induces somatic calli and SEs. But in rice, 0.125-0.5 mg/l MCPA is optimum for gynogenesis.
5. Other Factors:

   Sucrose level also appears to be critical. In sunflower, 12% sucrose leads to gynogenic embryo production, while at lower levels somatic calli and somatic embryos were also produced. Ovaries/ ovules are generally cultured in light, but atleast in some species, e.g., sunflower and rice, dark incubation favours gynogenesis and minimises somatic callusing. In rice, light may lead to degeneration of gynogenic proembryos.

Developmental Stages

Generally, gynogenesis has two or many stages and each stage may have distinct requirements. In rice, two stages, viz, induction and regeneration, are recognized. During induction, ovaries are floated on a liquid medium having low auxin and kept in dark, while for regeneration they are transferred on to an agar medium with higher auxin concentration and incubated in light.

Haploid plants generally originate from egg cell in most of the species (in vitro parthenogenesis) but in some species, e.g., rice, they arise chiefly from synergids; in atIeastAllium tuberosum even antipodals produce haploid plants (in vitro apogamy). As in anther culture, gynogenesis may occur either via embryogenesis or through plantlet regeneration from callus. In rice MCPA generally leads to a small amount of protocorm like callus formation from which shoots and roots regenerate, while picloram promotes embryo regeneration. In contrast, sugarbeet usually shows embryo development, while in sunflower embryos regenerate following a callus phase. In general, regeneration from a callus phase appears, at least for the present, to be easier than direct embryogenesis.

Limitations of Ovary Culture

Ovary culture has mainly two limitations:

i. so far it has been successful only in less than two dozen species, and

ii. the frequency of responding ovaries (1-5%) and the number of plantlets/ovary (1-2) is quite low.

iii. Therefore, anther culture is preferred over ovary culture. Only in those cases where anther culture fails, e.g., sugarbeet, and for male sterile lines, ovary culture assumes significance.

Embryo Culture

What do you understand by Embryo culture?

Embryo culture is the sterile isolation and growth of an immature or mature embryo in vitro, with the goal of obtaining a viable plant.

The first attempt to grow the embryos of angiosperms was made by Hannig in 1904 who obtained viable plants from in vitro isolated embryos of two crucifers Cochleria and Raphanus (Hannig 1904). In 1924, Dietrich grew embryos of different plant species and established that mature embryos grew normally but those excised from immature seeds failed to achieve the organization of a mature embryo (Dietrich, 1924). They grew directly into seedlings, skipping the stages of normal embryogenesis and without the completion of dormancy period. Laibach (1925, 1929) demonstrated the practical application of this technique by isolating and growing the embryos of interspecific cross Linum perenne and L. austriacum that aborted in vivo. This led Laibach to suggest that in all crosses where viable seeds are not formed, it may be appropriate

to excise their embryos and grow them in an artificial nutrient medium.

Embryo Culture is now a well-established branch of plant tissue culture.

There are two types of embryo culture:

i. Mature embryo culture:

It is the culture of mature embryos derived from ripe seeds. This type of culture is done when embryos do not survive in vivo or become dormant for long periods of time or is done to eliminate the inhibition of seed germination. Seed dormancy of many species is due to chemical inhibitors or acids, mechanical resistance present in the structures covering the embryo, rather than dormancy of the embryonic tissue.

ii. Immature embryo culture/embryo rescue:

It is the culture of immature embryos to rescue the embryos of wide crosses. This is mainly used to avoid embryo abortion with the purpose of producing a viable plant.

The underlying principle of embryo rescue technique is the aseptic isolation of embryo and its transfer to a suitable medium for development under optimum culture conditions. Florets are removed at the proper time and either florets or ovaries are sterilized. Ovules can then be removed from the ovaries. The tissue within the ovule, in which the embryo is embedded, is already sterile.

For mature embryo culture, either single mature seeds are disinfected or if the seeds are still unripe then the still closed fruit is disinfected. The embryos can then be aseptically removed from the ovules.

Utilization of embryo culture to overcome seed dormancy requires a different procedure. Seeds that have hard coats are sterilized and soaked in water for few hours to few days. Sterile seeds are then split and the embryos excised.

The most important aspect of embryo culture work is the selection of medium necessary to sustain continued growth of the embryo. In most cases, a standard basal plant growth medium with major salts and trace elements may be utilized.

Technique

Mature embryos can be grown in a basal salt medium with a carbon energy source such as

sucrose. But young embryos, in addition require different vitamins, amino acids, and growth regulators and in some cases natural endosperm extracts. Young embryos should be transferred to a medium with high sucrose concentration (8-12%); which approximate the high osmotic potential of the intracellular environment of the young embryosac, and a combination of hormones which supports the growth of heart-stage embryos (a moderate level of auxin and a low level of cytokinin).

Reduced organic nitrogen as aspargine, glutamine or casein hydrolysate is always beneficial for embryo culture. Malic acid is often added to the embryo culture medium. After one or two weeks when embryo ceases to grow, it must be transferred , to a second medium with a normal sucrose concentration, low level of auxin and a moderate level of cytokinin which allows for renewed embryo growth with direct shoot germination in many cases. In some cases where embryo does not show shoot formation directly, it can be transferred to a medium for callus induction followed by shoot induction. After the embryos have grown into plantlets in vitro, they are generally transferred to sterile soil and grown to maturity.

Organogenic Potential of Embryo Callus

An embryo callus is reported to possess a high regenerative capacity compared to those derived from mature organs, such as the leaf, stem and root. This is especially true of crucifers, cereals and millets. The age of the embryo considerably influences the regenerative ability of maize calluses. Green and Phillips (1975) obtained a differentiating callus from maize embryos excised 18 DAP. No differentiation occurred if the callus originated from a mature embryo. Immature embryos of oats, barley, sorghum, wheat, rice, rye, rye-grass and triticale are good explants for initiating callus capable of plant regeneration. The suspension cultures of Pennisetum americanum, known to differentiate whole plants from isolated protoplasts, were derived from immature embryos. To obtain a callus with morphogenetic potential excised immature embryos are placed on an agar medium with the scutellum facing up in the presence of 2,4-D alone or in combination with cytokinin. An embryo callus of Picea abies regenerated numerous shoot buds but only in one instance did the root differentiate (Hu and Wang 1986).

Applications

1. Prevention of embryo abortion in wide crosses: Successful interspecific hybrids have been seen in cotton, barley, tomato, rice, legume, flax and well known intergeneric hybrids include wheat x barley, wheat x rye, barley x rye, maize x Tripsacum, Raphanus sativus x Brassica napus. Distant hybrids have also been obtained via embryo rescue in Carica and Citrus species. Embryo rescue technique has been successfully used for raising hybrid embryos between Actidinia deliciosa x A. eriantha and A. deliciosa x A. arguata.

Resistance traits transferred to cultivated species through embryo rescue technique.

Crossing species	Resistance trait(s)
Lycopersicon esculentum x L.peruvianum	Virus, fungi and nematodes
Solanum melongena x S. khasianum	Brinjal shoot and fruit borer (Leucinodes arbonalis)
Solanum tuberosum x S. etuberosum	Potato leaf roll virus
Triticum aestivum x Thynopyrum scripeum	Salt tolerance
Hordeum sativumn x H. vulgare	Powdery mildew and spot blotch
Hordeum vulgare x H. bulbosum	Powdery mildew
Oryza sativa x O. minuta	Blast (Pyricularia grisea) and Bacterial blight (Xanthomonas oryzae)

2. Production of Haploids: Embryo culture can be utilized in the production haploids or monoploids. Kasha and Kao (1970) have developed a technique to produce barley

monoploids. Interspecific crosses are made with Horeum bulbosum as the pollen parent, and the resulting hybrid embryos are cultured but they exhibit H. bulbosum chromosome elimination resulting in monoploids of the female parent H. vulgare.

3. Overcoming seed dormancy: Embryo culture technique is applied to break dormancy. Seed dormancy can be caused by numerous factors including endogenous inhibitors, specific light requirements, low temperature, storage requirements and embryo immaturity. These factors can be circumvented by embryo excision and culture.
4. Shortening of breeding cycle: There are many species that exhibit seed dormancy that is often localized in the seed coat and/or in the endosperm. By removing these inhibitions, seeds germinate immediately. Seeds sometimes take up and give O_2 very slowly or not at all through the seed coat, and so germinate slowly if at all, e.g. Brussels sprouts, rose, apple, oil palm and iris. These (Ilex) are important plants for Christmas decorations. Ilex embryos remain in the immature heart-shaped stage though the fruits have reached maturity.
5. Prevention of embryo abortion with early ripening stone fruits: Some species produce sterile seeds that will not germinate under appropriate conditions and eventually decay in soil e.g., early ripening varieties of peach, cherry, apple, plum. Seed sterility may be due to incomplete embryo development, which results in the death of the germinating embryo. In crosses of early ripening stone fruits, the transport of water and nutrients to the yet immature embryo is sometimes cut off too soon resulting in abortion of the embryo. Eg: Macapuno coconuts. These are priced for their characteristic soft endosperm which fills the whole nut. These nuts always fail to germinate because the endosperm invariably rots before germinating embryo comes out of the shell. Embryo culture has been practised as a general method in horticultural crops include avocado, peach, nectarine and plum. Two cultivars 'Goldcrest peach' and 'Mayfire nectarine' have resulted from embryo culture and commercially grown.
6. Clonal Micropropagation: The regenerative potential is an essential prerequisite in nonconventional methods of plant genetic manipulations. Because of their juvenile nature, embryos have a high potential for regeneration and hence may be used for in vitro clonal propagation. This is essentially true of conifers and graminaceous members.

   Complete plantlets in vitro from conifers were achieved in long-leaved pine (Pinus palustris) through embryo culture (Sommer et al. 1975). This was followed by plantlet formation and their clonal micropropagation from embryos of P. elliottii, P. radiata, P. regida, P. monticola, P. taeda, P. virginiana and P. sabiniana. Cotyledonary buds were also induced in P. coulleri (Patel and Berlyn 1983) and P. strobus (Minocha 1980). Both organogenesis and somatic embryogenesis have been induced in major cereals and forage grasses from embryonic tissues. Generally, callus derived from immature embryos of cereals has the desired morphogenetic potential for regeneration and clonal propagation. As an exception, plant regeneration of orchid grass can be achieved in subcultured tissues derived from mature embryos. Probably these embryos are very small and the degree of development as well as organisation may be similar to that of immature embryos of large cereal grains.
7. Germination of seeds of obligatory parasites without the host is impossible in vivo, but is achievable with embryo culture.

   Plant embryo culture is an invaluable breeding technique as far as it is possible to synthesise hybrids from incompatible crosses. However, the number of hybrid seedlings rescued in many instances is extremely low due to the difficulty in growing very young embryos. Further viability decreases with age of the embryo in most of the incompatible crosses. Efforts are needed to identify the requirements for embryos of progressively younger stages in major crop species.

Conclusion

Ovary/ ovule and embryo cultures have found wide application in the production of haploid plants which are useful in plant breeding. An important aspect of plant breeding is the induction of maximum genetic variability of germplasm sources to secure a wider scope for selection and introduction of better trait qualities in existing crop species. Therefore, induction of androgenesis and gynogenesis have proved to be useful tools in the hands of plant breeders.

Questions

1. Discuss the factors affecting gynogenesis.
2. Briefly describe the technique of embryo culture under the following heads:
   a. nutritional requirements
   b. culture strategies
   c. environmental conditions for culture.
3. Write down the practical applications of ovary/ ovule and embryo culture.

Note